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THE MODE OF CHROMOSOME REDUCTION[1]

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In 1894 STRASBURGER (**22**), in his well-known paper in the *Annals of Botany*, placed the alternation of generations in plants on a chromosome basis, and showed that not only does a reduction of the chromosomes take place which marks the passage from sporophyte to gametophyte, but that the reduced or gametophytic number is phylogenetically the primitive number, the dominance of the sporophyte with the diploid number in the life cycle having been reached chiefly in the higher phyla of plants.

The stimulus to investigation resulting in part from that paper has led to two results: (1) the determination, for many members of each plant group, of the point in the life cycle where reduction occurs; and (2) the detailed investigation of the exact method by which the process of chromosome reduction is effected. The former line of activity has led to a clear understanding of the life cycle in nearly all plants, and is therefore of fundamental significance for plant morphology. The latter line of inquiry has led to the expression, during the last fifteen years, of a great variety of opinions concerning the precise nature of the reduction process, such opinions affecting fundamentally our conceptions regarding the nature of the chromosomes themselves and the part they play in hereditary processes. These cytological investigations, having gone forward simultaneously on plants and animals, have served to prove the fundamental unity and universality of meiotic phenomena in sexual organisms, so that the present-day cytologist

[1] The main features of this paper were presented by invitation before the National Academy of Sciences, St. Louis Meeting, November 8, 1910.

must take cognizance of the extensive literature on this subject concerning both plants and animals, and also the rapidly developing field of experimental cytology, in making his interpretations and drawing his conclusions. Nowhere is the fundamental unity of all organic matter better exemplified than in the study of these nuclear processes.

It is not my purpose to trace the history of the many changes of opinion regarding the method of chromosome reduction which have occurred as our knowledge of this process has developed. I wish merely to consider certain of the current views, and to express my own point of view, which has resulted from careful studies of reduction in various races and species of *Oenothera*, and the comparison with many other forms,[2] both from preparations and from the literature, as well as from a consideration of the many important data from experimental cytology, and the crossing of forms whose chromosomes differ in number or in morphology. This point of view, therefore, results from the consideration of data bearing on the chromosome question from every angle, so that it would be useless to attempt a citation of all the facts upon which it is based. Nor shall I attempt in this paper to formulate a complete hypothesis of chromosome behavior, nor of the precise hereditary rôle of the chromosomes. My special endeavor will be to show how the chief divergent current opinions regarding meiosis may be unified and harmonized. This will of course involve incidentally the expression of certain views regarding the nature of the chromosomes themselves. I regard it as the duty of every discoverer of new facts to bring them into relation, and if possible into harmony, with the other authenticated facts in the same field. The present paper is an attempt to fulfil this function with regard to my own studies on chromosome reduction. To do this in the present state of our knowledge of this process requires that the subject be approached from a broader viewpoint, if the

[2] I am greatly indebted to Professor GRÉGOIRE for kindly giving me the use of his laboratory facilities during my stay at Louvain, and for many animated and critical discussions of current cytological problems. I am also indebted to Professor STRASBURGER for the courtesies of his laboratory, and for the privilege of examining a large number of cytological preparations. I alone, however, am responsible for the views here expressed.

different methods described are to be welded into one. Perhaps it may also be hoped that the viewpoint here developed will help to stimulate some of the further investigations on this fascinating subject.

Much of the confusion and change of opinion with regard to meiotic phenomena have come from the study of different stages of the process at different times. The earlier investigators of this subject devoted their attention almost entirely to the study of the heterotypic chromosomes or "tetrads," and the manner of distribution of the elements of which each was composed. It was chiefly in later studies that the necessity for determining the manner of origin of the heterotypic chromosomes was realized, so that the studies of the last five or six years have been directed mainly to an understanding of the earlier stages of meiosis, from the telophase of the last premeiotic mitosis to diakinesis. These include the leptonema, zygonema, pachynema, and strepsinema stages (names, some of which involve different interpretations), all of which GRÉGOIRE (**12**, p. 239) prefers to include under the general term synapsis. The synizesis, a special name proposed by MCCLUNG (**13**) for the stage when the delicate chromatic threads occupy but a small part of the nuclear cavity, is also included in this period. In this paper I shall use the term synapsis in its more restricted and more usual botanical sense, as equivalent to synizesis.

The later stages of meiosis, from diakinesis onward, are now pretty clearly understood and agreed upon by most cytologists, particularly those who have studied plant forms. It is the events of the earlier stages which are still in dispute. Some of the most useful contributions of the most recent papers have been with regard to the earliest stages of all, from the "resting" reticulum of the spore mother cell to the synizesis condition.

The use of the term "tetrads" as applied to the heterotypic chromosomes by nearly all the earlier students of meiosis, both in plants and animals, has led to much confusion, due to the fact that these bodies exhibit a great variety of forms and appearances, and in many cases are not tetravalent at all, but bivalent or gemini, and are not due to the split of a single body, but to the approxi-

mation of two somatic chromosomes which may or may not show fission. The attempt to interpret bivalent structures as "tetrads" has been a frequent source of error. The significance of the term "tetrad" as applying to the heterotypic chromosomes was of course derived from the supposed theoretical implications, derived from WEISMANN'S distinction between a longitudinal or quantitative, and a transverse or qualitative division of the chromosomes. It was assumed that if the heterotypic chromosomes gave the appearance of being tetrads or tetravalent structures, then they must have originated from two segmentations, one of which was longitudinal of the spirem and the other transverse. This conception has since lost much of its usefulness, and this is a case where the too close adherence to a useful and stimulating theory has tended finally to retard progress. While such a difference is not impossible, it has not yet been shown by critical observation or experiment that there is any fundamental distinction between a longitudinal and a transverse segmentation of a chromosome. But, on the other hand, many facts with regard to chromosome continuity or individuality of a certain type must be regarded as well established. Longitudinal fission of all the chromosomes is regarded as universal in somatic mitoses, and this has frequently been pointed to as a strong argument in favor of the view that a chromosome is composed of qualitatively different portions or bodies arranged along its long diameter, whose equal division and distribution it is the function of this longitudinal fission to bring about. But it may be pointed out that this longitudinal fission of the viscous chromosome bodies may be determined by purely physical forces resulting, for example, from the electrical charges carried by the chromatin particles.

If this were the case, instead of an equal distribution of "ids" to each daughter chromosome, longitudinal fission would mean merely fission for mechanical or physical reasons along whatever becomes the longitudinal axis of the chromosome as it changes from the reticulate condition of the resting nucleus to the compact condition of prophase or metaphase. There is no observational evidence that longitudinal fission means any more than this, nor that in the passage from the alveolate or reticulate condition of the

resting nucleus to the compact condition of the prophase, any particular arrangement of differential units of structure composing an individual chromosome takes place.

Studies of forms whose chromosome group is composed of bodies morphologically unlike (heteromorphic) have shown, for example in various insects, that the chromosomes frequently maintain the same space relationships to each other in each equatorial plate, and therefore also probably during the intervening "resting" conditions of the nuclei. Other clear evidence, given, for example, by BOVERI (1 and 1*a*), leads to the same conclusion. Hence it is probable that the direction of the long axis of the chromosomes, and hence their plane of division, correspond in successive mitoses. It does not therefore follow, from the statements of the last paragraph, that there is no difference between a longitudinal and a transverse division of a somatic chromosome. For even though such a transverse fission may be of no hereditary significance in separating unlike parts of a chromosome, yet it may have an important mechanical significance, and as far as the morphology of the product is concerned, the result of a transverse split of certain chromosomes in a nuclear plate may very well be different from that of a longitudinal fission. STRASBURGER (**25**, p. 437) suggests as an explanation of the heteromorphic chromosomes in such forms as *Funkia*, *Yucca*, and *Galtonia* that the short chromosomes have arisen by the transverse segmentation of certain of the long chromosomes. This may have been a consequence of the differentiation of the parts of such chromosomes, or may have resulted from more purely mechanical causes, but at any rate it furnishes no evidence that successive longitudinal segments of the long chromosomes which maintain their unity are unlike.[3] Therefore, although, phylogenetically considered, transverse segmentations of members of the chromosome group have doubtless occurred in various species, it does not follow that these segmentations have any fundamental hereditary significance,

[3] In a few forms, such as *Ascaris*, having very few chromosomes, they appear to be compound structures, as shown by their fragmentation in somatic mitoses; but in the great majority of forms no evidence of the compound character of the chromosomes is forthcoming.

because the products of the segmentation all remain in the same nucleus and are propagated in each mitosis.

There is, therefore, no satisfactory cytological evidence that the chromosomes are composed of smaller units whose equal division and distribution is brought about in each mitosis. According to the most careful cytological studies, we cannot with any assurance affirm the existence of smaller differential units composing the chromosomes. On the other hand, the many lines of evidence indicating the more or less independent behavior and genetic continuity of the chromosomes within a nucleus from mitosis to mitosis in the vast majority of cases, seems clear and incontrovertible. To the writer, this independence of behavior will find an explanation in some difference, probably of a chemical nature, between the materials of which the individual chromosomes are composed.

That this is insufficient, however, as an entire explanation, seems to be shown by the case of *Oenothera gigas* (GATES **6**). This mutant has 28 chromosomes, double the number found in its parent, *O. Lamarckiana*. As stated in the above-mentioned paper, *O. gigas* probably contains merely a duplicate set of *O. Lamarckiana* chromosomes, although other changes seem to have occurred simultaneously in producing the mutation. The new number of chromosomes persists, however, and this shows that even though certain of the chromosomes are as much alike as two chloroplasts, yet, having occurred in a given nucleus, they will reappear in its descendants. This may be accounted for by the fact that the mitotic mechanism brings about a simultaneous division of all the chromosomes present. But it does not account for the further fact that all these bodies reappear in the prophase of each mitosis, and hence must have maintained their identity in some way while in the alveolated and distributed condition of the resting nucleus.

The lines of evidence which favor this conception of chromosome continuity from mitosis to mitosis are too numerous to enumerate, and since this view in some form is now widely accepted by cytologists, an enumeration is unnecessary in the present connection. One clear result showing the unity in behavior of the chromosomes was found in the hybrid *O. lata*×*O. gigas* (**5**). This hybrid had 21 chromosomes, 7 derived from the *O. lata* egg and

14 from the *O. gigas* male cell. In reduction they segregated regularly into groups of 10 and 11. The recent work on animal species and hybrids with heteromorphic chromosomes, by BOVERI, BALTZER, HERBST, TENNANT, and others, including the remarkable cases in which certain chromosomes are extruded, is all a confirmation of the same point of view.

We have thus reached the view that, while the chromosomes clearly behave as more or less independent units of structure, we are not justified by observational evidence in assuming that they in turn are composed of smaller morphological units. Further, what has frequently been interpreted as a transverse segmentation of the spirem or of the meiotic chromosome gemini is now known to be, in most cases at least, only the separation of whole somatic chromosomes. It may therefore be questioned whether a transverse fission of the chromosomes themselves ever regularly occurs. As already pointed out, however, this may be for purely mechanical or physical reasons.

From this point of view let us now examine the question of the method of chromosome reduction. In former papers (**4, 5, 7**) I have shown that reduction in *Oenothera* takes place according to the FARMER and MOORE method of telosynapsis, the spirem segmenting into a chain of chromosomes arranged end-to-end. In the heterotypic mitosis these (somatic) chromosomes, which may or may not be visibly in pairs, are segregated into two groups at the poles of the spindle. Each of these chromosomes undergoes a longitudinal split during the anaphase or telophase of the heterotypic mitosis, and the halves thus produced are distributed by the homotypic mitosis. The essential and critical stages which show that this is the method of reduction in *Oenothera* were presented in my paper of 1908. The suggestion of GRÉGOIRE, in his very useful summary of the literature of chromosome reduction (**12,** p. 325), that a strepsinema stage had been omitted between the pachynema and diakinesis, cannot apply. It is evident from an examination of figs. 18–32 in my paper already referred to (**4**) that such a stage cannot be intercalated. Especially figs. 18, 22, 23, and 24 leave no room for any other interpretation than the obvious one that a pachynema thread is segmenting into a chain

of chromosomes. Nothing but the exigencies of a theory would tempt anyone to suggest any other explanation. GEERTS (10), afterward confirmed by DAVIS (2), has reached the same conclusion.

I therefore regard it as certain that in *Oenothera* the pachynema segments directly into a chain of chromosomes arranged end-to-end. That one can reach such a degree of certainty on this point is largely due to the peculiarly favorable character of these critical stages in *Oenothera*, the chromosome number being small (14) and the chromosomes themselves being relatively short and stout, so that the difficulties attending the interpretation of the long threadlike chromosomes are not encountered here.

While, therefore, an end-to-end arrangement or telosynapsis of the chromosomes occurs in *Oenothera*, I think there is also adequate evidence of a side-by-side pairing, or parasynapsis, in certain other forms. I have presented this point of view in several papers (**4, 5, 7**), and have been further confirmed in it by my studies in other laboratories.

It is not necessary to specify here all the plants which are demonstrably telosynaptic, and those which are believed to be demonstrably parasynaptic, but a few of the cases which are best established may be cited. Among recent accounts, in addition to *Oenothera*, telosynapsis in plant forms seems to have been adequately shown by YAMANOUCHI (**28**) in *Fucus*. The most convincing recent accounts of parasynapsis have been by GRÉGOIRE (**11**) with figures of *Lilium*, *Osmunda*, and *Allium;* ROSENBERG (**18**) for *Drosera;* and YAMANOUCHI (**27**) for *Nephrodium*. In *Galtonia*, STRASBURGER first (**23**) gave a telosynaptic account. Later, MIYAKE (**14**) in STRASBURGER'S laboratory decided for parasynapsis. In certain of the later stages, *Galtonia* evidently resembles *Oenothera*. MIYAKE'S figures (pl. 3, figs. 23–32) indicate the segmentation of a pachynema into a chain of chromosomes. It was only after long search, as MIYAKE states (p. 96), that stages as represented by figs. 17–19 were found, indicating a lateral pairing of long narrow chromosomes (strepsinema). According to the ordinary method of cytological interpretation, he concludes that the latter stage must be intercalated, and that all the chromosomes always pass through the strepsinema stage of his fig. 18. This

interpretation assumes an unwarranted amount of "secondary fusion" between the chromosomes to form a chain, as indicated, for example, in his figs. 23 and 24. It seems to the writer equally justifiable to assume that the apparent rarity of a strepsinema stage is real, and that usually the chromosomes do not pass through such a stage during maturation. This obviates the necessity of finding an explanation for an amount of "secondary fusion" between the ends of the chromosomes, which is not easily accounted for. This has always been a serious stumbling-block for those who affirm the universality of parasynapsis, in which the pachynema is followed by a strepsinema stage.

Let us now inquire what is the exact difference involved between the methods of telosynapsis and parasynapsis as described in current papers. Telosynapsis involves the folding and looping and subsequent segmentation of a single thick thread (pachynema) which may have previously exhibited a split which closed up, to reappear only in the anaphase or telophase of the heterotypic mitosis as a longitudinal split in the components of the heterotypic gemini. Parasynapsis involves the lateral pairing of delicate threads at a stage earlier than the pachynema, their more or less complete fusion to form the pachynema, and their subsequent separation to form the strepsinema stage with paired threads, which, by shortening and thickening and (in the cases where a continuous pachynema or strepsinema spirem is supposed to be formed) transverse segmentation to form chromosome pairs, which when first formed are characteristically composed of two long and narrow threadlike chromosomes lying side-by-side and more or less coiled about each other. Later, by shortening and thickening, these chromosomes may, as in *Drosera* (ROSENBERG **18**), become short and stout, or as in *Lilium* they may remain relatively long and narrow, even in diakinesis and the following metaphase.

I think it can be shown upon analysis that the difference between these two methods of reduction is far less fundamental than has been generally supposed.[4] I further believe that the

[4] A recent important paper by Miss DIGBY (**2***a*) is quite in accord with this point of view. Miss DIGBY has made a fresh and careful study of somatic and meiotic divisions in *Galtonia*. She interprets the paired threads in early prophase of the

importance and significance of the synapsis stage, even though this condition is unique in the life cycle, has been greatly overestimated; and that the difference between the prophase of an ordinary somatic mitosis and that of the heterotypic mitosis (that is, the presynaptic and postsynaptic stages, from the resting reticulum of the last somatic mitosis to diakinesis) is much less fundamental in character than generally assumed in the current literature. The main fact of reduction, according to the most careful studies of either the telosynaptic or the parasynaptic method, is that in the heterotypic mitosis, instead of dividing, the chromosomes (which represent whole somatic chromosomes) segregate. Nor is there any adequate evidence that the mechanism of meiosis is meant to accomplish anything more than this. The reduction divisions have been studied without sufficient comparison with somatic mitoses, and it is probable that various features of the postsynaptic stages will be found to be devoid of any greater significance than attaches to corresponding spirem stages in the prophases of ordinary mitoses. Theories based on a supposed interchange of "chromomeres" or other materials during certain stages of the heterotypic prophase can be cast aside as having no cytological foundation in critical observation. The most careful recent studies have failed, not only to find any evidence for an interchange of chromomeres, but even to substantiate the idea that the chromatic threads are composed of linin in which chromatin granules are imbedded. The careful observations of GRÉGOIRE and others have served to show that the threads are composed of one general material, the varying density or alveolation of which may give the appearance of granules.

heterotypic mitosis as representing the two edges of a single alveolated chromosome, and therefore equivalent to the similar paired structures in any somatic prophase. The apparently greater definiteness of the zygonema threads, however, would perhaps indicate that the structures represented in the two cases are not necessarily always the same. Regarding the synaptic pairing she says: "The important point which *Galtonia* demonstrates is that its spireme is univalent. Whether these univalent strands join with their homologous pairs telosynaptically or parasynaptically, or by any other intermediate method between these two extremes, resolves itself merely into a question of non-essential detail." Since *Galtonia* is a form having both long and short chromosomes, this perhaps accounts for the great variety observed in the method of pairing. Possibly if the individual chromosomes can be followed it may be found that the longer ones are more likely to pair parasynaptically and the short ones telosynaptically.

Others, for example OVERTON (**17**), have suggested that even though there is no exchange of bodies between the paired threads of the presynapsis, yet the purpose of this pairing may be to bring the threads within each other's "influence." A little reflection, I think, will show the futility of this idea. In *Oenothera* the nuclei of the pollen mother cells have an average diameter of less than 10 μ. The diameter of the presynaptic threads may be taken to be not more than 0.27 μ. Supposing the presynaptic threads to come to lie within their own diameter of each other, it is not clear what chemical or other "influence" could be exerted at the latter distance, which could not be exerted at the former. When it is considered that the chromosomes of the synaptic nuclei have, in higher plants, gone through hundreds of thousands of divisions since they were first associated at the time of fertilization, and that between every two divisions were periods in which the chromosomes were in the alveolated and distributed condition of the "resting" nucleus, in which the portions of the reticulum representing each chromosome must come into the most intimate contact, at least at their boundaries; and when it is further considered that during all this time active metabolic interchanges between nucleus and cytoplasm are taking place, the idea that a pairing of chromosomes or threads at synapsis is necessary for an exchange of influences loses its force.

This leads to another series of facts which students of reduction have frequently failed to take sufficiently into account, namely, that in somatic mitoses the homologous chromosomes are in pairs. MONTGOMERY (**15**) first suggested, in 1901, that in reduction homologous chromosomes of maternal and paternal origin pair. In the following year SUTTON (**26**) showed that in *Brachystola magna*, in which various shapes of chromosomes occur, those of like shape are paired in the spermatocytes. The same thing has since been shown for many other animals, and also for various plants. In 1905 STRASBURGER (**24**) found that this paired condition is not confined to the heterotypic or synaptic chromosomes, but occurs also in the somatic tissues. This was shown by studies of *Galtonia* and *Funkia*, in which the chromosomes are heteromorphic,

being of different lengths. The same thing has since been found to be the case in several other plants having morphological differences in their chromosome group. GEERTS (9) published two figures indicating that in *Oenothera*, in which the chromosomes exhibit no morphological differences (that is, are isomorphic), they are also in pairs in the somatic tissues. Two figures (9 and 10) in a subsequent paper of mine (GATES 5) show indications of the same thing. Therefore, though this cannot be so clearly demonstrated in organisms whose chromosomes are isomorphic, yet it cannot be doubted that the chromosomes are in homologous pairs throughout the somatic cells. OVERTON (17) gives indications of this in *Thalictrum* (pl. 1, fig. 1), and believes (p. 45) that the homologous parental elements are finally brought side-by-side in the somatic nuclei. CLEMENS MÜLLER (16) has recently given a particularly clear demonstration of this paired arrangement, from studies on the root tips of *Yucca* species, in which the chromosomes are either very long or very short.

From these results it is evident that the pairing of homologous chromosomes is not brought about at synapsis or any other period of meiosis, but that the chromosomes are really paired throughout the life cycle of the sporophyte. The pairing must therefore have taken place at the time of fertilization. One of the best contributions that could be made to the study of the life cycle would be the determination of just how the two single sets of x chromosomes in the egg and sperm nuclei become, in the nuclei descended from the fertilized egg, a set of $2x$ chromosomes arranged in homologous pairs. The idea that the final act of fertilization, that is, the pairing of homologous chromosomes, is deferred until synapsis, an idea which has been often expressed, is therefore a mistaken one, and views of synapsis and its importance in the life cycle will have to be modified accordingly. The view that the function of synapsis is to bring about a pairing of chromosomes or of spirems is no longer justified, (1) because the chromosomes are now known to be paired throughout the somatic tissues of the sporophyte, (2) because there is no satisfactory evidence of a smaller unit of structure within the chromosomes whose union or exchange could be brought about if the materials were stretched out into slender

parallel threads, and (3) because even if there were such units they would have an equally good or even better opportunity for interchange during the alveolated reticulum stage which always intervenes between somatic mitoses.

From this point of view, the life cycle of any sexual plant or animal (with reservations for the Ascomycetes and other groups where peculiar conditions of sexuality occur) may be outlined as follows: At or soon after fertilization, the materials composing the sets of chromosomes of the egg and sperm nuclei become arranged in pairs, so that in subsequent mitoses throughout the sporophyte or soma they always reappear as pairs of homologous chromosomes, the members of which originated respectively from the egg and the sperm or male cell. Synapsis plays no special part in the pairing, and indeed appears occasionally to be omitted in some forms.[5] Meiosis or reduction consists essentially in the segregation of the members of these pairs which have been in association since soon after fertilization. This segregation is followed immediately by what is essentially another mitosis. The gametophytic or germ cell nuclei may then continue to divide with the haploid number of chromosomes until the diploid number is restored by fertilization. The chromosomes are therefore in pairs from the time of fertilization onward, and the members of the pairs are merely segregated in the heterotypic mitosis. The completion of the act of fertilization is not deferred until synapsis, but takes place probably soon after the union of the sexual nuclei; and throughout the sporophyte or soma the chromosomes maintain, to some extent, their relative space relations with each other.

This leaves several obvious points unexplained. Why are there almost universally *two* meiotic divisions without a growth period of the chromosomes between them? The conception of

[5] GRÉGOIRE (12, p. 332) says regarding synapsis: "Le ramassement synaptique ne peut avoir, par lui-même, aucun rôle à jouer dans l'accomplissement des phénomènes de réduction, mais doit être considéré plutôt comme une conséquence des phénomènes essentiels qui se déroulent dans le noyau. Cela résulte de ce que, dans certains objets, on ne retrouve pas le ramassement synaptique (SCHREINER, JANSSENS, DETON) et que néanmoins les stades leptotènes, pachytènes (et même zygotènes) y montrent une évolution absoluement identique à celle que l'on constate dans les objets où se manifeste un ramassement."

BOVERI (1) answers this question. After each mitosis, the chromosomes, and also the nuclei and cytoplasm, must grow before they divide again. Otherwise they will all continue to diminish in size. In tissues whose cells are of approximately equal size, the chromosomes must grow to their original size after each mitosis. At the beginning of meiosis, the chromosomes undergo the usual growth, so that, although the individuals are separated into two groups in the heterotypic mitosis, they must still divide again immediately before they are in condition to undergo further growth. GRÉGOIRE (**12**, p. 383) therefore suggests that the heterotypic mitosis is a process of chromosome separation intercalated between the last and the next to the last division of the diploid generation.

If the mere separation of the chromosomes whose ancestors have been in close association throughout the sporophyte is the function of meiosis, then the peculiarly characteristic phenomena of synapsis are without an explanation. In a paper on reduction in *Oenothera* (**4**), I referred to the size relationships of the cells and nuclei in the archesporium and the pollen mother cells. It was pointed out (p. 5) that the cells and nuclei of the sporogenous tissue continue to grow simultaneously from the size indicated by figs. 1 and 2 to that indicated by fig. 4 of that paper. Then (p. 7) the pollen mother cells cease to grow, while their nuclei continue enlarging. This is shown by comparing figs. 4 and 13. During this later growth of the nuclei, synapsis occurs. In figs. 4–10 of the paper cited, the nuclei are all of similar size and are taken from presynaptic cells in the condition shown by fig. 4. In all the later figures (12–32), which are during or after synapsis, the nucleus is seen to be conspicuously larger. A comparison of fig. 4 with figs. 12 and 13 makes it evident that the synaptic "contraction" is partly only an appearance, due to the sudden growth of the nucleus, that is, an increase in the amount of the karyolymph. Occasional threads remain attached to the nuclear membrane and are drawn outward as the nucleus enlarges (cf. figs. 12 and 13). There is also some contraction, however, as shown by comparing the diameter of the reticular area in figs. 12 and 13 with that of the nucleus in fig. 4. In fig. 15, which represents a typical "synaptic ball," it is also evident that the diameter of this ball is less than that of the

nucleus in fig. 4, and it is equally evident that the threadwork in the synaptic ball is much denser than that in the reticulum of fig. 4. Undoubtedly the most important thing that is going on at this time is a rearrangement of the threads of the reticulum to form the more or less continuous threadwork of synapsis. But similar rearrangements go on in the prophase of every somatic mitosis. The peculiar appearance is given to synapsis, and in some degree to the subsequent stages up to diakinesis, by the enormous growth of the nuclear cavity; but, as already pointed out, there is also a certain amount of contraction as the threads of the reticulum become transformed into those of the synaptic ball. In the paper cited I interpreted synapsis in the ordinary way, as resulting from a contraction, but it is evident that this only partly explains the phenomenon, which is largely due to an increase of the karyolymph, accompanied by a rearrangement, without any growth, of the chromatic threads.[6] It is to be hoped that future students of reduction will make careful series of measurements, to see whether the same size relationships hold for other forms. In making such measurements it will be necessary to take account of the fact that in the later stages of synapsis the nuclear membrane frequently becomes extremely delicate or practically disappears, allowing the cytoplasm to encroach on the nuclear

[6] In a conversation with Dr. LAWSON at Brussels during the Botanical Congress, he first suggested to me that the synaptic appearance is due to a growth of the nucleus rather than a contraction of the nuclear contents. He has since kindly sent me an advance proof of his paper (12*a*), the appearance of which was unfortunately delayed, so that I might make more extended reference to it. In studies of the pollen mother cells of *Smilacina* at the time of synapsis, he finds no contraction whatever of the chromatin. From a series of measurements of the presynaptic and synaptic nuclei in *Oenothera*, I find, as above stated, a slight amount of contraction in the area occupied by the nuclear reticulum (though this probably has no special significance), but a very large growth of the nucleus without a corresponding amount of growth in the cytoplasm. Our results, therefore, are essentially in agreement. LAWSON attributes the growth of the nucleus in the pollen mother cell to the fact that the latter is charged with food materials, which leads to the disappearance of vacuoles from the cytoplasm, and an accumulation of sap within the nuclear cavity. The fact that synapsis is now known to be almost coextensive with sexuality itself, occurring even in Myxomycetes (OLIVE), would seem to call for a more general explanation of the phenomenon. Later in the present paper I have suggested the direction in which it seems probable to the writer that the explanation of the nuclear growth without chromatin growth, which causes the synaptic appearance, is to be found.

area. Measurements should therefore be made only on nuclei whose membrane is distinct; and of course where nuclei are cut by the knife, only sections should be measured which pass through their greatest diameter.

The fact that, at the beginning of the so-called synapsis period, a conspicuous growth of the nucleus of the pollen mother cell takes place, without an appreciable growth in the size of the cell, means that there must be at this period a readjustment in the Kern-plasma relation (HERTWIG). In another paper (**6**) considerable attention was devoted to this matter of the Kernplasma relation in *O. gigas*.

It is known that in all higher plants the pollen mother cells first undergo a large amount of growth, in which the nucleus and cytoplasm share simultaneously. In *Oenothera*, as here stated, and probably also in many other plant and animal forms, the nucleus then undergoes further growth, while the cytoplasm remains stationary. The earlier growth of the nucleus of the pollen mother cell is accompanied by a corresponding growth in its chromatic content, so that the reticulum continues to fill the nuclear cavity; but its later growth is due merely to an increase in the karyolymph, while the amount of chromatin ceases to grow, and the reticulum therefore ceases to occupy the whole of the nuclear cavity. During this later nuclear expansion, the chromatin begins the series of rearrangements which change the reticulum to the spirem condition and finally to diakinesis, and which do not necessarily differ in any fundamental particular from the prophases of any somatic mitosis. The peculiar appearances at this time, as compared with somatic prophases, are partly the result of the fact that the rearrangements go on in a much larger cavity, which allows the chromatic materials to be more loosely distributed; while the peculiarities of the diakinesis and the heterotypic gemini may be partly accounted for by the fact that the members of the pairs, instead of lying parallel, usually occupy a great variety of positions relative to each other. Furthermore, in many forms the attraction between homologous chromosomes is probably greater at this time than in somatic mitoses, for the heterotypic gemini are often more closely paired than the chromosomes during the sporophytic divisions.

However, this is not invariably true, and I have shown that in *Oenothera Lamarckiana* and its mutants, and in *O. biennis* (**4** and **5**, p. 183), the diakinetic chromosomes are usually very loosely paired, owing to a weak attraction between homologous chromosomes.[7] In *O. grandiflora*, as I have already pointed out (**7**), the figures of DAVIS (**2**) indicate that this attraction is greater than in the other forms.

It is not to be supposed that these suggestions offer a full explanation of all the phenomena, but they deserve to be carefully tested in future studies of synapsis. The nuclear enlargement unaccompanied by cytoplasmic growth is probably connected with the fact that two subsequent mitoses, without further chromatin growth, follow each other.

A recent paper by STOMPS (**21**), on reduction in *Spinacia*, offers some particularly accurate figures of the presynaptic stages (pl. 1, figs. 7–13), drawings of which have been so frequently more or less diagrammatic. STOMPS finds that the chromosomes are never joined to form a continuous spirem, but that their free ends can always be determined. There are 12 somatic chromosomes in *Spinacia*, and in the presynaptic nuclear reticulum STOMPS believes he is able to determine definitely 6 elongated threadlike darker-staining bodies, which are not joined end-to-end, but variously arranged. Each of these bodies is considered to be composed of two longitudinal portions more or less completely fused, which are interpreted as representing two chromosomes laterally paired. These threads are so delicate that interpretations are extremely difficult, so that the correctness of this interpretation must depend upon the accurate demonstration of the number of these bodies. In later stages the threads are very much shorter and thicker, but the figures (pl. 2, figs. 6–13) do not form a close enough series to show whether the final chromosome bivalents are formed by a longitudinal or a transverse segmentation of the 6 (?) bodies represented in the presynaptic stages. If the author's

[7] In December 1908, in a paper read before the Botanical Society of America (Further studies of Oenotheran cytology; abstract in *Science* **29**:269. 1909), I showed that the phenomena of reduction in *O. biennis* and in *O. laevifolia* agree in every detail with my earlier account (**4**) of that process in *O. Lamarckiana* and its mutants.

interpretation is correct, then we have here, somewhat as in ROSENBERG'S account for *Drosera*, a lateral pairing of long thread-like chromosomes, which afterward by contraction become short and thick.

It has sometimes been urged as an argument for the parasynaptic method of reduction, that the chromosomes are in pairs side-by-side in the somatic mitoses. When, as is very generally the case in somatic mitoses, the long axis of the chromosomes is several times their short axis, then for mechanical reasons, if they are to continue to keep together in pairs, they will naturally lie side-by-side in the crowded somatic nuclear prophase and metaphase. The same is true of *Oenothera* (as already referred to in this paper), in which the somatic chromosomes are from three to six times longer than broad. They are paired side-by-side in the equatorial plate of somatic divisions. It is not yet known whether in prophase they lie paired side-by-side or end-to-end on a spirem, though the latter is probably the case. But in any case, they are clearly arranged end-to-end in the stages of reduction preceding diakinesis, though after the looped and folded chain segments, they frequently come to lie paired side-by-side. Such pairs, as I have pointed out in previous papers, are almost invariably joined by a linin connection at *one* end, showing clearly their manner of origin. This all goes to prove that while both end-to-end and side-by-side pairings of chromosomes occur, yet no great significance attaches to the difference. In the case of the heteromorphic chromosomes figured by C. MÜLLER (**16**) in root tips of *Yucca*, the long chromosomes lie in pairs side-by-side, but in the practically globular chromosomes both axes are of the same length, and the distinction between a lateral or endwise pairing breaks down.

Miss STEVENS (**20**) has shown that in one of the mosquitoes (*Culex*) "parasynapsis of homologous chromosomes often changes to telosynapsis in the metaphase of the first spermatocyte." She says (p. 216): "It is of especial interest to see in *Culex* a perfectly clear case of parasynapsis change in some cases to an equally clear case of telosynapsis before metakinesis, while intermediate ring stages and cases of overlapping ends also occur."

The general point of view resulting from the foregoing studies and considerations may now be briefly stated. Both the telosynaptic and the parasynaptic methods of reduction occur, but the difference is not of phylogenetic significance, depending rather upon the mechanics of nuclear processes. In forms having long threadlike chromosomes, the pairing may be expected to take place side-by-side and a strepsinema stage is therefore likely to occur, while with forms having short stout chromosomes, the pairing is, on account of the different spatial relations, more likely to be end-to-end, the pachynema segmenting directly into a more or less continuous chain of chromosomes. In the same form (for example, in *Oenothera*) the somatic chromosomes may be laterally paired in metakinesis, while the heterotypic gemini are at first arranged end-to-end, later frequently swinging round so as to lie side-by-side. It is probably true, as already pointed out, that the chromosomes in somatic prophases in this genus are also at first arranged endwise. This requires further investigation.

It is also evident that the difference between parasynaptic and telosynaptic pairing in meiosis is devoid of hereditary significance, for reasons already stated. Since the chromosomes are in homologous pairs from the early divisions of the fertilized egg onward, the need for a synaptic contraction to bring about an exchange of particles or influences is imaginary. The synaptic contraction is instead (at least in some forms) in large part an appearance, due to an inordinate increase in the karyolymph at this time. The main established facts regarding the life cycle are that the chromosomes are in homologous pairs throughout the sporophyte, and that the members of the pairs are segregated in the heterotypic mitosis.

I may call attention to the fact that, although reduction consists simply in segregation of the descendants of homologous chromosomes which were first associated as pairs soon after fertilization and remained so associated throughout the sporophyte or soma, yet the orientation of these bodies in the heterotypic metakinesis permits varying distributions of the respective mater-

nal and paternal chromosomes during reduction. This is of particular interest from the standpoint of hybrids, but will not be discussed further in this connection.

A striking proof that a redistribution of characters occurs in sexual but not in asexual or vegetative reproduction is to be found in the case of potatoes. The recent experiments of EAST (**3**) and of SALAMAN (**19**) show that potato varieties grown year after year from tubers usually continue true. But when the flowers are self-pollinated the first generation of offspring may show plants of different types. Thus a race of potato with red tubers may on self-pollination produce sexual offspring some of which bear only red tubers and others only white. Here is direct evidence that a segregation and redistribution takes place in sexual reproduction which is absent in vegetative reproduction, or occurs only in the rare cases of "bud sports," through the loss of a character.

The chances for chromosome redistribution during the process of segregation in the heterotypic metaphase certainly furnish the most probable basis for this segregation and redistribution of characters. But as I have pointed out elsewhere (**8**, p. 211) from the evidence of hybrids, this redistribution of characters must also occur in certain cases at other points in the life cycle.

Summary

Studies of chromosome reduction in different plants indicate that there are two general methods of reduction in organisms, one involving a telosynapsis or end-to-end arrangement of the chromosomes, the other involving a parasynapsis or side-by-side pairing. The difference, however, is not of phylogenetic significance, because both methods may occur in different genera of the same family; nor is it of hereditary significance, because the whole chromosome must be regarded as the unit of nuclear morphological structure.

In general, genera having short chromosomes will show telosynaptic pairing, while in forms with long threadlike chromosomes the chromosomes are likely to pair parasynaptically. In organisms having heteromorphic chromosomes, both methods of pairing may occur in the same nucleus. Whether the pairing shall be end-to-end or side-by-side is therefore not of phylogenetic or hereditary

importance, but is merely a matter of cell mechanics; and the two methods of chromosome reduction are therefore essentially one.

While the behavior of the chromosomes affords abundant evidence of some type of individuality or genetic continuity, yet there is no satisfactory evidence of any smaller unit of structure within the chromosome, and for this and other reasons there can be no hereditary difference between a parasynaptic and a telosynaptic pairing of chromosomes.

The one essential and probably almost universal fact of meiosis or reduction in sexual organisms is the segregation of whole somatic homologous chromosomes in the heterotypic mitosis. The reduction process is everywhere the same in involving a segregation of the somatic chromosomes in the heterotypic mitosis, and a split of these chromosomes in the homotypic.

Since it is now known that the chromosomes are in homologous pairs throughout the tissues of the sporophyte, this pairing must take place soon after the association in the fertilized egg of the two sets of chromosomes derived respectively from the egg and sperm nuclei.

The fact that these homologous chromosomes are closely associated in pairs throughout the sporophyte, deprives synapsis of its supposed function of bringing about an interchange of materials or "influences" just before the chromosomes finally separate.

If, as seems evident, the essential fact of meiosis is the mere segregation and redistribution of the chromosomes whose ancestors have been associated in pairs throughout the sporophyte, then the phenomena of the heterotypic prophase do not differ essentially from those of any somatic prophase.

The unique condition of synapsis or synizesis is considered to be due, in some forms at least (for example, in *Oenothera*), to a sudden growth in the nucleus of the pollen mother cell without corresponding growth of the cytoplasm or of the nuclear reticulum. There appears also to be some contraction of the chromatic threads in the nucleus at this time, but the most important change is a rearrangement of the threads from the reticular to the spirem condition.

The conspicuous appearance of "emptiness" of the synaptic

nucleus is largely due to its sudden expansion by an increase in the karyolymph. This involves a sudden change in the karyoplasmic relation, which is probably connected with the fact that, without further chromatic or cytoplasmic growth, two mitoses, one of which involves a segregation and the other a split of the chromosomes, take place in quick succession.

Synapsis, therefore, has no special significance in the life cycle, but depends upon a temporary change in the karyoplasmic relation, which is necessitated by the segregation process, intercalated between two mitoses, one of which (in plants) is sporophytic and the other gametophytic.

Since the life cycle involves a pairing of homologous chromosomes at the time of fertilization and their continued association in pairs until they are separated in the heterotypic mitosis, synapsis is not the delayed and final act of fertilization, as frequently interpreted. Conceptions of synapsis as bringing about an interchange of chromomeres or particles in the chromosomes are not supported by critical observations; and ideas involving an exchange of "influences" are rendered superfluous by the fact that the homologous chromosomes are paired throughout the sporophyte.

Reduction does not consist in a transverse or qualitative and a longitudinal or quantitative split of the chromosomes according to the conception of WEISMANN, but involves merely a segregation and redistribution of the members of homologous pairs of whole somatic chromosomes. If the most widely accepted general account of reduction be universal, then a transverse segmentation of the chromosomes never regularly occurs. But it may be for purely physical reasons that a somatic chromosome always splits longitudinally. It is not necessary to assume that the function of this split is to produce an equal division and distribution of differentiated "ids" arranged along its axis.

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